

Aus der Abteilung für klinische Immunologie des Universitätskrankenhauses
Erlangen-Nürnberg
(Vorstand: Professor Dr. med. F. Scheiffarth)

Immunologische Studien zur Entwicklung der experimentellen Hepatitis bei thymektomierten Tieren

Inaugural-Dissertation
zur
Erlangung der Doktorwürde
der
Hohen Medizinischen Fakultät
der
Friedrich-Alexander-Universität
zu
Erlangen-Nürnberg

vorgelegt von
Hans Joachim Schmidt
aus Stuttgart

1967



Dekan: Professor Dr. med. K. G. Ober

Referent: Professor Dr. med. F. Scheiffarth

Tag der mündlichen Prüfung: 13. September 1967

**International Archives of
Allergy and Applied Immunology**

Editors-in-Chief: R. R. A. COOMBS, Cambridge – H. C. GOODMAN, Geneva – F. HAHN, Freiburg
im Br. – D. HARLEY, London – P. KALLOS, Helsingborg – F. MILGROM, Buffalo, N.Y. – G. B.
WEST, Carshalton, Surrey
S. KARGER – BASEL/NEW YORK (Printed in Switzerland)
SEPARATUM

Int. Arch. Allergy 32: 308–317 (1967)

Immunological Studies on the Development of Experimental Hepatitis in Thymectomized Mice

F. SCHEIFFARTH, H. WARNATZ and H. J. SCHMIDT

Department of Clinical Immunology of the University Erlangen-Nürnberg

The activity of the lymphoid tissue is the prerequisite for specific immune responses. It is responsible for the production of humoral antibodies as well as for transplantation immunity and for delayed hypersensitivity reactions. Since the investigations of MILLER, it has been established that the thymus plays an important role in the development of an immune-competent lymphatic system (13–20, 22, 28). Postnatally thymectomized animals produce circulating antibodies against various antigens only in a low titer (1, 3, 7–9, 18, 19, 21, 23), but they do not reject homografts and the hypersensitivity to tuberculin is also impaired (2, 12, 15–18). Mice thymectomized immediately after birth finally succumb to the so-called wasting syndrome (16, 18, 20, 21, 25).

The role of the lymphatic system has been demonstrated in several autoimmune diseases (5, 4, 11), for instance, in experimental hepatitis of the mouse (26, 29). After sensitization of mice over a prolonged period with homologous liver extract mixed with Freund's adjuvant, histological damage of the liver was induced and this showed a similar morphological picture as chronic hepatitis does in man. Following transfer of lymphocytes from sensitized mice, liver damage of a similar kind was observed in the recipients. ³H-thymidine labelled lymphocytes of sensitized mice were detected in increased numbers in autoradiograms of the liver obtained from the recipients. On the basis of these experiments, it is likely that lymphocytes are of great importance for the development of the experimental hepatitis.

This work was supported by research grant Sche 36/8 of the 'Deutsche Forschungsgemeinschaft'.



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41533917_0030

The present experiments have attempted to evaluate the significance of thymectomy for the development of experimental hepatitis induced by sensitization with homologous liver extract mixed with Freund's adjuvant. Further experiments were carried out to determine whether the transfer of lymphocytes from adult isologous mice with experimental hepatitis could induce liver damage in thymectomized mice and non-thymectomized controls.

Methods

Animals and Technique of Thymectomy

Litters of at least 5 new-born mice of the inbred strain C57Bl were used. One half of each litter was thymectomized within the first 24 h according to the technique of MILLER. After cutting the skin and the sternum of the animals, the lobes of thymus were removed by suction with a pipette. The wound was sutured by atraumatic silk without bleeding and the treated mice were returned to their mothers. Within the first 5 days, 20–25% of thymectomized mice died either from surgery or neglect or cannibalism on the part of the mothers. The successful thymectomy was certified by continuous weight controls (Fig. 1). Thymectomized mice showed in the first 3 weeks a similar increase in weight as the untreated mice of the same litter. Thereafter, the rate of increase in body weight declined and in many instances body weight dropped a few days before death. On the average the animals died 4–10 weeks after birth with the signs of wasting syndrome (Fig. 2). The loss in body weight was accompanied by the morphological changes of the lymphatic organs, as described in the literature. Histological changes of the liver were not observed in thymectomized mice and in the controls between the 4th and 6th week after birth. However, in animals with advanced wasting syndrome (loss of hairs, shaggy coat and cowering posture), an infiltration of mononuclear cells in

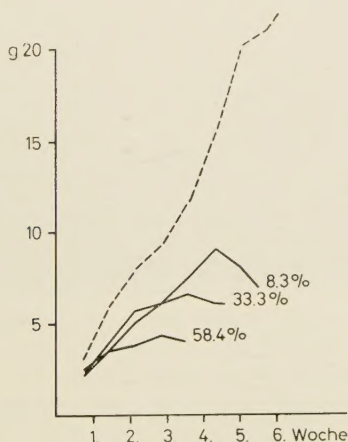


Fig. 1. Weight curves of thymectomized animals (—) and non-thymectomized controls (----) of the inbred strain C57Bl.

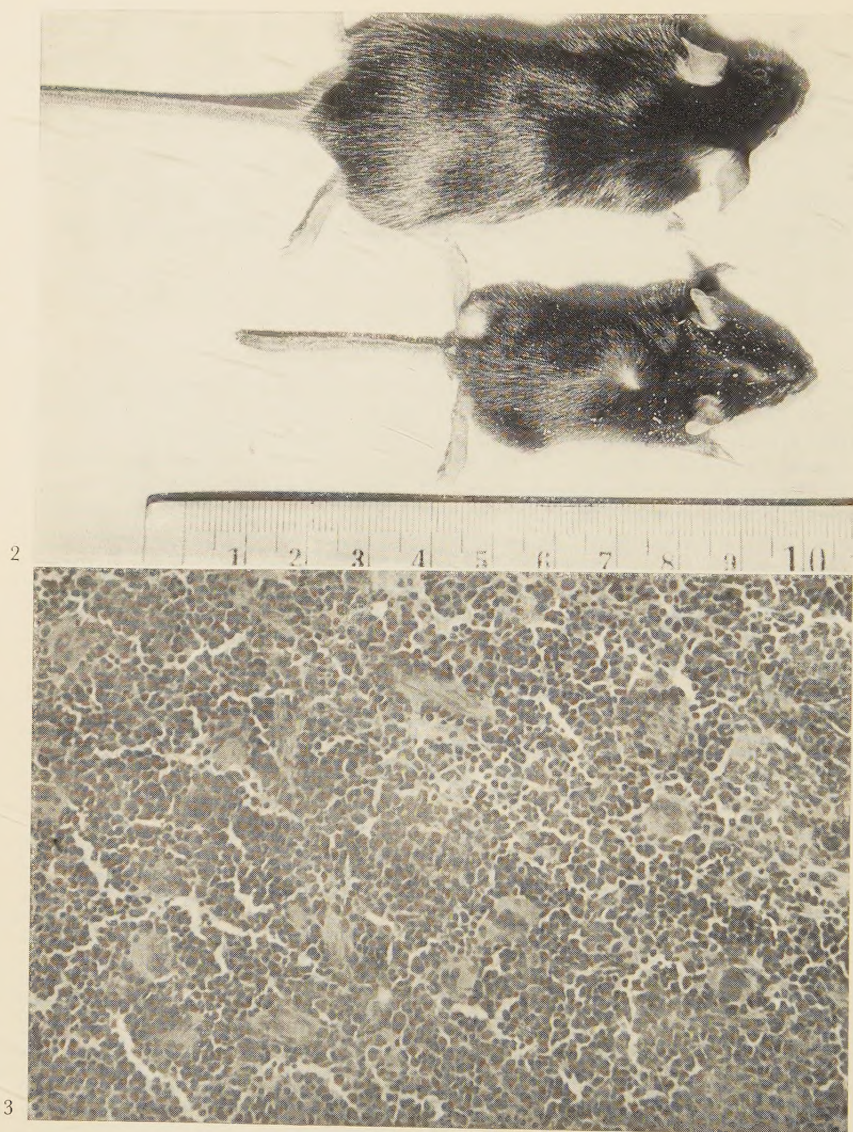
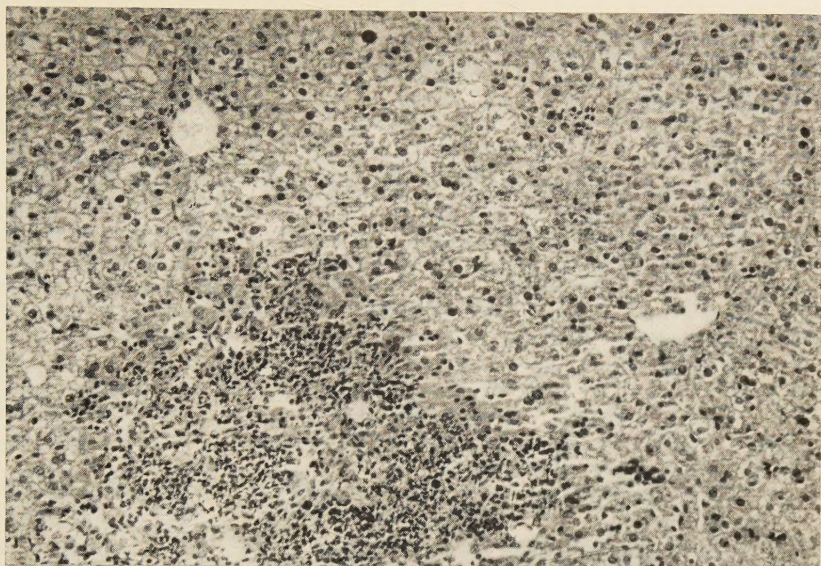


Fig. 2. A thymectomized mouse (bottom) and a non-thymectomized control (top) of the same litter 6 weeks after birth.

Fig. 3. Histological section of the spleen obtained from a thymectomized animal, with decrease of lymphocytes and follicles as well as an increase in megacaryocytes.

the liver was demonstrated. For demonstration of tolerance of the thymectomized mice skin-homotransplantation was performed. The survival time of skin from mice of the inbred strain AKR was longer than four weeks; in most animals the grafts were macro-



4



5

Fig. 4. Histological section of the liver of a thymectomized animal, sensitized against homologous liver extract mixed with Freund's adjuvant. These liver changes are evaluated as ++.

Fig. 5. Histological section of the liver of a thymectomized mouse 7 days after transfer of lymphocytes from adult isologous mice sensitized against homologous liver extract mixed with Freund's adjuvant. The liver changes are evaluated as +.

scopically intact at the time of death. Our investigations in the thymectomized mice as described below were carried out between the 2nd and 5th week after birth; in this time the symptoms of wasting syndrome were not fully developed.

Sensitization

Sensitization of thymectomized and control mice was begun 14 days after birth. Each animal received 5 intramuscular injections of 0.05 ml extract of homologous liver extract mixed with Freund's adjuvant in a ratio of 5:1, at intervals of two days. The liver extract was prepared by homogenization with a Potter-Elvehjem homogenizer of homologous livers obtained from untreated AKR mice. The last injection was given intraperitoneally without Freund's adjuvant, and the animals were sacrificed 10 days later. The success of thymectomy was proven by autopsy. Animals in which parts of thymus were found were not used in these experiments. Spleens of the thymectomized mice were examined histologically; the characteristic decrease in lymphocytes and follicles as well as an increased number of megacaryocytes were found (Fig. 3). In 3% of the animals, an adjuvant arthritis was observed. The livers of the thymectomized animals and the controls were removed and histological sections of the livers prepared. The changes in the livers were judged as follows:

Ø no histological alteration, + focal and diffuse increase of Kupffer cells and infiltration of periportal fields with mononuclear cells, ++ intensive focal or diffuse increase of Kupffer cells with necrosis of parenchyma cells (Fig. 4, 5).

In control series, some animals were injected with Freund's adjuvant alone, emulsified in physiological saline; others were injected with homologous liver extract without Freund's adjuvant in corresponding doses.

Transfer Experiments

Thymectomized and control mice were injected intravenously with lymphocytes from adult isologous mice sensitized against homologous liver mixed with Freund's adjuvant on the 14th day after birth. For sensitization, adult mice of the inbred strain C57Bl received 10 intramuscular injections of 0.1 ml liver homogenate from the AKR strain mixed with Freund's adjuvant in a ratio 5:1 over a period of 3 weeks. The animals were sacrificed on the 10th day after the last injection. Liver damage was evaluated according to the above-mentioned scale. In 50% of these animals, liver sections showed a profound damage (++), in 44.5% a moderate liver damage (+) and in 5.5% no detectable changes. Livers of the control animals treated in the same manner with Freund's adjuvant alone showed moderate damage in 55.5% and no liver damage in the rest. Immediately after sacrificing the animals, lymph nodes and spleens were removed, freed from the surrounding connective tissue and shredded. The cell suspension consisted mainly of large, medium and small lymphocytes. The percentage of polymorphonuclear cells was below 5%. Cell counts were made and the viability rate of the transferred cells determined with the nigrosin test. About 95% of the lymphocytes were viable, i.e. were not dyed black by nigrosin.

The cell suspension was injected into the tail vein of the thymectomized and the non-thymectomized mice 14 days after birth at a dose level of $15-20 \times 10^6$ cells. After 7 or 14 days, the animals were sacrificed and the livers were examined histologically.

Results

The histological results from thymectomized and control mice after sensitization with homologous liver extract mixed with

Table I

Distribution of histological liver damage in the thymectomized animals and in the non-thymectomized controls after sensitization with homologous liver extract mixed with Freund's adjuvant (FA), with Freund's adjuvant alone, or with liver extract alone.

Animals	Antigen	Histological liver damage		
		Ø	+	++
Thymectomized mice	homologous liver + FA	24	16	13
	FA alone	15	3	
	homologous liver alone	12	1	
Non-thymectomized controls	homologous liver + FA	13	4	20
	FA alone	14	7	
	homologous liver alone	14	1	

Ø no histological liver damage

+ diffuse or focal increase of Kupffer cells and infiltration of the periportal fields with mononuclear cells.

++ intensive diffuse and focal infiltration of the liver with mononuclear cells and necrosis of parenchyma cells.

Freund's adjuvant are summarized in Table I. This shows that 24.5% (13/53) of the thymectomized animals had profound liver damage and 30.2% showed moderate liver damage after sensitization; in the non-thymectomized controls, profound liver damage was observed in 54.0% (20/37) of the animals and moderate damage in 10.8%. Hence, in the non-thymectomized mice a profound liver injury was induced more frequently.

The results of cell transfer experiments are summarized in Table II. After transfer of lymphocytes from animals sensitized against homologous liver, profound liver injury was not found. Only moderate liver changes with focal and diffuse increase in Kupffer cells were observed and one week after cell transfer the liver changes in the thymectomized mice were as frequent as in the controls. After two weeks, however, more animals in the control group showed liver changes, and in addition, the changes were more intensive. Mice which received lymphocytes of isologous mice treated with Freund's adjuvant showed moderate liver damage in several histological sections, but these quickly regressed in thymectomized animals. In the controls, the liver changes were found in a high percentage of animals up the 10th day after cell

Table II

Distribution of histological liver damage in thymectomized animals and non thymectomized controls after transfer of lymphocytes from adult isologous mice treated with liver extract mixed with Freund's adjuvant (FA), with Freund's adjuvant alone, or with lymphocytes of untreated animals. The results were obtained 7 or 14 days after cell transfer.

Animals	Transfer of lymphocytes from	Day	Histological liver damage		
			Ø	+	++
Thymectomized mice	mice sensitized against homologous liver + FA	7	11	9	0
		14	15	5	0
	mice treated with FA alone	7	11	9	0
		14	18	2	0
	untreated mice	7	12	1	0
		14	10	0	0
Non-thymectomized mice	mice sensitized against homologous liver + FA	7	12	8	0
		14	6	13	1
	mice treated with FA alone	7	11	9	0
		14	10	9	0
	untreated mice	7	4	1	0
		14	5	0	0

transfer. Controls receiving lymphocytes of normal mice exhibited no liver changes.

Discussion

These experiments confirm earlier reports that liver changes resembling the morphological picture of chronic hepatitis in man can be induced in mice by long-term sensitization with homologous liver extract mixed with Freund's adjuvant. Unlike the chronic hepatitis in man, however, this experimental hepatitis is not progressive. The mice recover in 3-5 weeks after completion of sensitization (26, 29). This liver damage cannot be transferred to untreated animals by serum; in other words, it cannot be induced by circulating antibodies (27). Intravenous transfer of lymphocytes from liver damaged mice into isologous normal mice induces, however, an experimental hepatitis in the recipients which is similar to that of the donor animals (26). In autoradiographic studies, the transferred lymphocytes were seen in increased numbers in the livers of the recipients and this strongly suggests that these lymphocytes have a special affinity to liver tissue.

In thymectomized mice, liver injury can be induced by sensitization with homologous liver extract mixed with Freund's adjuvant as well as by transfer of lymphocytes from liver-damaged

animals. This implies that the development of experimental hepatitis with its increase in Kupffer cells and its infiltration of the periportal fields is not influenced by thymectomy. The intensity and the frequency of these liver changes were lower in thymectomized animals than in the non-thymectomized controls. These findings are contradictory to other immunological phenomena which cannot be produced in thymectomized animals, such as rejection of homologous grafts, delayed hypersensitivity reactions or allergic encephalomyelitis. It should be mentioned that the so-called adjuvant arthritis (2) was more frequent in thymectomized animals than in the controls. In our experiments, arthritis was found in about 5% of the thymectomized mice whereas no joint disease was seen in the controls using the same dosage of Freund's adjuvant.

The transfer of lymphocytes from sensitized mice produced a moderate liver damage in 50% of thymectomized animals and controls. But whereas in the controls the frequency increased two weeks after cell transfer, the picture of the liver obtained from the thymectomized mice was nearly normal again. This finding is striking inasmuch as it should be assumed on the basis of MILLER's experiments (16-18) that the transferred lymphocytes show a stronger immunological reactivity in the thymectomized than in the control mice. The changes in the livers of thymectomized mice have the same morphological characteristics as those of the controls. So in both groups hemopoietic centres in the liver were observed in the first two weeks after birth. The liver injury caused by the wasting syndrome (20) show significant differences from the liver changes induced either by sensitization or by transfer of lymphocytes from liver-damaged animals. In the wasting syndrome, a focal necrosis of liver parenchyma cells was always found.

Summary

After sensitization against homologous liver extract mixed with Freund's adjuvant, liver damage was induced in about 50% of neonatally thymectomized mice which showed a similar morphological picture as chronic hepatitis does in man. The intensity and frequency of these liver changes were lower in thymectomized animals than in control animals of the same age. Following transfer of $15-20 \times 10^6$ lymphocytes obtained from liver damaged isologous animals, moderate liver damage occurred in neonatally thymectomized recipients as well as in non-thymectomized controls.

References

1. ARCHER, O. K.; PIERCE, J. C.; PAPERMASTER, B. W. and GOOD, R. A.: Reduced antibody response in thymectomized rabbits. *Nature, Lond.* 195: 191 (1962).
2. ARNASON, B. G. and JANKOVIČ, B. D.: Suppression of delayed hypersensitivity reactions in rats thymectomized at birth. *Fed. Proc.* 21: 274 (1962).
3. ARNASON, B. G.; JANKOVIČ, B. D. and WAKSMAN, B. H.: Effect of thymectomy on 'delayed' hypersensitive reactions. *Nature, Lond.* 194: 99 (1962).
4. ARNASON, B. G.; DE VAUX ST. CYR, C. and GRABAR, P.: Immunoglobulin abnormalities of the thymectomized rat. *Nature, Lond.* 199: 1199 (1963).
5. BURNET, F. M.: Autoimmune diseases - experimental and clinical. *Proc. roy. Soc. Med.* 55: 619 (1962).
6. BURNET, F. M. and MACKAY, I. R.: Lymphoepithelial structures and autoimmune disease. *Lancet* ii: 1030 (1962).
7. HARRIS, T. N.; RHOADS, J. and STOKES, J. A.: A study of the role of the thymus and spleen in the formation of antibodies in the rabbit. *J. Immunol.* 58: 27 (1948).
8. HUMPHREY, J. H.; PARROTT, D. M. V. and EAST, J.: Studies on globulin and antibody production in mice thymectomized at birth. *J. Immunol.* 92 (1964).
9. JANKOVIČ, B. D.; WAKSMAN, B. H. and ARNASON, B. G.: Role of the thymus in immune reactions in rats. I. The immunologic response to bovine serum albumin (antibody formation, Arthus reactivity, and delayed hypersensitivity) in rats thymectomized or splenectomized at various times after birth. *J. exp. Med.* 116: 159 (1962).
10. LOUITT, J. F.: Immunological and trophic functions of lymphocytes. *Lancet* ii: 1106 (1962).
11. MACKAY, I. R. and BURNET, F. M.: Autoimmune Diseases. Pathogenesis, Chemistry and Therapy (Thomas, Springfield 1963).
12. MARTINEZ, C.; KERSEY, J.; PAPERMASTER, B. W. and GOOD, R. A.: Skin homograft survival in thymectomized mice. *Proc. Soc. exp. Biol., N.Y.* 109: 193 (1962).
13. METCALF, D.: The effect of thymectomy on the lymphoid tissues of the mouse. *Brit. J. Haemat.* 6: 324 (1960).
14. METCALF, D.: The thymus and lymphopoiesis; in R. A. GOOD and A. GABRIELSEN's Proceedings of the Conference on the Thymus, 1963.
15. MILLER, J. F. A. P.: Immunological function of the thymus. *Lancet* ii: 748 (1961).
16. MILLER, J. F. A. P.: Role of the thymus in transplantation tolerance and immunity in transplantation; in G. E. W. WOLSTENHOLME and M. P. CAMERON's Ciba Found Symp., p. 384 (Churchill, London 1962).
17. MILLER, J. F. A. P.: Role of the thymus in transplantation immunity. *Ann. N.Y. Acad. Sci.* 99: 340 (1962).
18. MILLER, J. F. A. P.: Effect of neonatal thymectomy on the immunological responsiveness of the mouse. *Proc. roy. Soc. B* 156: 415 (1962).
19. MILLER, J. F. A. P.: Effect of thymic ablation and replacement; in R. A. GOOD and A. GABRIELSEN's Proceedings of the Conference on the Thymus, 1963.
20. MILLER, J. F. A. P. and DUKOR, P.: Die Biologie des Thymus nach dem heutigen Stande der Forschung (Akademische Verlagsgesellschaft, Frankfurt a. M. 1964).
21. MILLER, J. F. A. P.; MARSHALL, A. H. E. and WHITE, R. G.: The immunological significance of the thymus. *Adv. Immunol.* 2: 111 (1962).
22. NAKAMURA, K. and METCALF, D.: Quantitative cytological studies on thymic lymphoid cells in normal, preleukaemic and leukaemic mice. *Brit. J. Cancer* 15: 306 (1961).
23. PAPERMASTER, B. W.: Significance of the thymus in the evolution of the lymphoid tissue and acquired immunity; in R. A. GOOD and A. GABRIELSEN's Proceedings of the Conference on the Thymus, 1963.

24. PAPERMASTER, B.W.; CONDIE, R.M. and GOOD, R.A.: Immune-response in the California hagfish. *Nature, Lond.* 196: 355 (1962).
25. PARROTT, D. M. V.: Strain variation in mortality and runt-disease in mice thymectomized at birth. *Transplant. Bull.* 29: 102 (1962).
26. SCHEIFFARTH, F.; WARNATZ, H. and MAYER, K.: Studies concerning the importance of mononuclear cells in the development of experimental hepatitis. *J. Immunology* 98: 396 (1967).
27. SCHEIFFARTH, F.; WARNATZ, H. und NIEDERER, W.: Tierexperimentelle Untersuchungen zur Pathogenese der chronischen Hepatitis. I. Morphologische Studien an der Leber nach Sensibilisierung mit homologen Leberzellfraktionen. *Virchows Arch. path. Anat.* 339: 358 (1965).
28. WAKSMAN, B. H.; ARNASON, B. G. and JANKOVIČ, B. D.: Role of the thymus in immune reactions in rats. III. Changes in the lymphoid organs of thymectomized rats. *J. exp. Med.* 116: 187 (1962).
29. WARNATZ, H.; SCHEIFFARTH, F.; WOLF, F. and SCHMIDT, J. H.: Autoradiographic experiments concerning the importance of mononuclear cells in experimental hepatitis. *J. Immunology* 98: 402 (1967).

Authors' address: Drs. F. Scheiffarth, H. Warnatz and J. H. Schmidt, Dept. of Clinical Immunology, University of Erlangen-Nürnberg, University Hospital, 8520 Erlangen (Germany).

LEBENS LAUF

Am 9. Juni 1942 wurde ich, Hans Joachim S c h m i d t, als Sohn des Zahnarztes Dr. rer. pol. et Dr. med. dent. Hans Joachim Schmidt und seiner Ehefrau Wilma, geb. Hügelschäffer, in Stuttgart geboren.

Von 1948 bis 1952 besuchte ich die Filderschule in Stuttgart-Degerloch und anschließend die Oberrealschule in Stuttgart und bestand dort 1961 die Reifeprüfung.

Ab Sommersemester 1961 studierte ich an der Universität Erlangen-Nürnberg Zahnmedizin. Am 20.11. 1964 legte ich die zahnärztliche Vorprüfung ab. Im Sommersemester 1967 begann ich mit dem Zahnmedizinischen Staatsexamen.

